Mutations in the Genes Regulating Methylene Tetrahydrofolate Reductase (MTHFR C–>T677) and Cystathione ß-Synthase (CBS G–>A919, CBS T–>C833) Are Not Associated with Myocardial Infarction in African-Americans

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Running Head: CBS and MTHFR genes in **African-American** MI patients

ABSTRACT

Moderate hyperhomocysteinemia is a putative risk factor for cardiovascular disease. Molecular studies have demonstrated increased plasma homocysteine levels in the presence of DNA mutations in either methylene tetrahydrofolate reductase (MTHFR) enzyme found in the remethylation pathway or the enzyme cystathione B-synthase (CBS) of the transsulfuration pathway. To determine whether the mutation C÷T677 in the MTHFR gene or the T÷C833/844ins68 and G÷A919 mutations in the CBS gene are associated with myocardial infarction (MI) in African-Americans, DNA was analyzed from samples obtained from a case-control study conducted at a large, inner-city hospital. One-hundred ten African-American subjects with a diagnosis of MI and 185 race and age matched controls were recruited. Our results demonstrated that 15% of the MI cases were heterozygous for the C÷T677 (MTHFR) mutation while 1.8% were homozygous. When compared to the controls in which 15% were heterozygous and 2.1% were homozygous, no significant association with MI was observed. In addition, 34% of the cases were heterozygous for the T÷C833 (CBS) mutation while 6% were homozygous. This compared to 32% and 5% of the controls having the heterozygous and homozygous genotype, respectively. No significant association was observed for the T÷C833 (CBS) mutation among the cases and controls. Although this mutation has no significant association with MI, the prevalence of the heterozygous state was higher than what has been reported for whites (12%). No mutations for G÷A919 (CBS) were detected in the cases or controls. The racial differences of the CBS T–>C833 polymorphism suggest that further investigation into the other areas of the CBS gene is needed.

Key Words: myocardial infarction, African-Americans, homocysteine,MTHFR gene, CBS gene

INTRODUCTION

Both genetic and acquired abnormalities in either the methylenetetrahydrofolate reductase (MTHFR) enzyme found in the remethylation pathway or the cysthionine B-synthase (CBS) enzyme found in the transsulfuration pathway can lead to hyperhomocysteinemia.[1,2] Hyperhomocysteinemia has been recognized as an important risk factor for both venous and arterial disease.[3,4,5] While it is known that plasma determinations of homocysteine levels are important for the diagnosis and management of hyperhomocysteinemia, identification of DNA mutations in genes that encode enzymes responsible for homocysteine metabolism are important for the development of rapid screening methods that can be used to identify those who may be at risk for developing cardiovascular disease.

In the remethylation pathway, the C –> T677 variant represents a common mutation in the MTHFR gene that results in an alanine-to-valine substitution.[1] Associations between this variant in the absence of documented hyperhomocystemina and occlusive vascular disease have been controversial. While Morita et al[6] have reported an association between coronary artery disease and homozygosity for the variant in Japanese subjects, Wilcken et al[7] found no such association among Australians. It has been suggested that these differences may be reflective of the different ethnicities.[6] In the U.S., several recent studies have reported the prevalence of this mutation to be higher in Caucasians than in African-Americans.[8,9,10], but few data are available concerning the relationship between occlusive vascular disease and this mutation in African-Americans.

In the transsulfuration pathway, several DNA polymorphisms which may be linked to cardiovascular disease have also been identified. A T to C transition at nucleotide position 833 (T–>C833) is a highly prevalent mutation in the CBS gene found in homocytinuric patients with decreased CBS enzyme

activity.[11,12,13] Based on a birth prevalence in Denmark, it has been estimated the homozygosity of this variant among the Danish is 1:20,500.[14] However there have been reports suggesting that the prevalence of the CBS T–>C833 mutation may vary among populations as well as within certain geographic regions.[15] In tandem with the T–>C833 mutation, a 68-base pair insert (844ins68) has also been identified in exon 8.[11,16] The presence of this insert has added to the complexity of the genetic regulation of homocysteine metabolism.[17] Although it was initially thought that this insertion introduced a premature termination codon that would result in a non-functional protein[12], it was later shown that this insertion actually corrected the T->C833 mutation through the creation of an alternative splice site.[16,19] Other reports have shown that homocysteine levels in subjects who either fasted or were methionine loaded were lower in individuals who had the 68-base pair insert as compared to those without the insert.[19] It was further reported that this decrease was only seen in individuals who had low Vitamin B6 levels thus suggesting that the effect of the insertion was associated with low concentrations of pyridoxial-5-phosphate . Although several studies have found a non-significant increase in the 833 variant in subjects with occlusive vascular disease, the association of the T->C833/844ins68 mutation with coronary artery disease is nevertheless unclear.[20,21]

In this same pathway, a G to A transition at nucleotide 919 (G–>A919) represents another mutation in the CBS gene.[22] This mutation, highly prevalent in homocystinuric patients of Celtic origin, has been associated with pyridoxine nonresponsiveness.[23] Other studies have also reported that the frequency of the G->A919 mutation may vary among populations.[11]

Although it is clear that the prevalence of mutations in the MTHFR and CBS genes can differ among populations, it is unclear what impact these differences may have on the prevalence and pathogenesis of cardiovascular disease. The purpose of the present study is to explore the genotype frequencies of the

C÷T677 mutation in the MTHFR gene and the T÷C833 and G÷A919 mutations in the CBS gene and their relationship with myocardial infarction among African-Americans.

MATERIALS AND METHODS

Cases were selected from outpatients age 65 or younger attending the cardiology clinic for follow-up management of myocardial infarction at a large, urban public hospital in Atlanta, Georgia in 1995-1996. Controls were selected from outpatients attending a clinical laboratory for routine blood tests at the same hospital and were frequency matched to cases on age (within 10 years), sex, and race. Control subjects with a history of myocardial infarction, stroke, or blood clots were considered non- eligible. The consent rate for both cases and controls was over 90%. The present analysis is limited to those cases form the cardiology clinic with a diagnosis of myocardial infarction.

Participation in the study entailed granting permission to review medical records, an in-person interview, and the collection of 20 ml of blood. The questionnaire elicited information on basic demographics, lifestyle habits, a personal history of thrombosis and other medical problems, and a family history of blood clots, stroke, or heart attack.

Further examination of the mutations of interest was performed on cord blood samples collected from consecutive births at an Atlanta hospital during 1997-1998. Ethnicity information was collected with each newborn sample.

Laboratory Methods

The presence of zygosity for the polymorphisms of the MTHFR and CBS genes were determined from DNA extracted from a blood sample. Blood samples were collected in 0.109 M sodium citrate. DNA was extracted from 3 ml of whole blood using a Gentra DNA extraction kit (Minneapolis, Minnesota) per the manufacturer's's instructions and stored at -20^{N}C. Polymerase chain reaction was used to amplify DNA fragments in the genes. The MTHFR C677T was amplified as previously described.[1]

Primers for 833T->C/844ins68 were 5' CTGCCTTGAGCCCTGAAGCG-3'(forward) and 5' ACCGTGGGGATGAAGTCGcGAG-3' (reverse). The products were amplified for 35 cycles of 95 C denaturing for 1 minute, 65 C annealing for 1 minute, and 72 C extension for 2 minutes. The PCR products were then run on an ethidium bromide-stained 1% agarose gel, fragments were extracted, purified and then subsequently fluorescently sequenced with an internal primer. Confirmatory sequencing was necessary due to interference of the 68 base-pair insertion following restriction enzyme digestion. A subset of samples was selected at random and confirmed by direct nucleotide sequencing. Quality control for the DNA analyses was maintained by the use of both positive and negative controls in each set of analyzed samples, and results were confirmed independently by two different laboratory workers.

Statistical Methods

The MTHFR genotypes are denoted as C/C (no mutation), C/T (heterozygous for the mutation), and T/T (homozygous for the mutation). The CBS genotypes for the T÷C833 polymorphism are denoted T/T (no mutation), T/C (heterozygous for the mutation), and C/C (homozygous for the mutation) and for G÷A919 polymorphism are denoted G/G (no mutation), G/A (heterozygous for the mutation), and G/G (homozygous for the mutation). Odds ratios were used as a measure of association between zygosity of the genes and myocardial infarction and were obtained using Mantel-Haenszel techniques.[24] All reported p-values are two-tailed, and 95% confidence intervals are reported.

RESULTS

The distribution of cases and controls according to selected characteristics is displayed in Table 1. The mean age of cases and controls was 56 and 55 respectively. Forty-four percent of the cases and 51% of the controls are women. Cases were more likely to have a history of hypertension (86% compared to 66%), to have a family history of vascular disease (66% compared to 43%) and to have a history of smoking habit (82% compared to 55%).

Forty-six MI cases and 171 controls were tested for the CBS G–>A919 mutation and no mutations were found. Analysis for this mutation among these cases and controls was curtailed at this point. Analysis of 152 African-American and 111 Caucasian newborn samples revealed no mutations for the CBS G–>A919 polymorphism.

In Table 2 the odds ratios relating myocardial infarction and MTHFR C–>T677 and CBS T–>C833 are displayed. No association between the heterozygous or homozygous state of the MTHFR C–>T677 polymorphism and myocardial infarction was observed. A recessive allele model (C/C vs. C/T and T/T) yielded an odds ratio of unity (odds ratio = 1.0, 95 percent confidence interval 0.53-1.87; p value = 0.9). The C/T genotype was found in 15% of cases and controls. The prevalence of the T/T genotype was very low in cases and controls (1.8% and 2.2% respectively).

Similarly, neither the heterozygous nor the homozygous genotype of the CBS T–>C833/844ins68 was associated with myocardial infarction. A recessive allele model (T/T vs. T/C and C/C) yielded an odds ratio close to unity (odds ratio = 1.1, 95 percent confidence interval 0.69-1.86; p value = 0.6). The T/C genotype was found in approximately 33% of cases and controls. Eight controls (4.6%) and 7 cases (6.5%) possessed the homozygous genotype. The prevalence of the heterozygous T/C genotype among

80 African-American and 94 Caucasian newborn samples was 37.5% and 13.8%, respectively. The homozygous C/C genotype was present in 2.5% of the African-American newborns, and not present among the Caucasian newborns.

DISCUSSION

The designation of any genetic polymorphism/mutation as a candidate gene for a genetic risk factor for a given disease is generally based on its prevalence in controls and cases. Since genetic polymorphisms vary among populations, it is important to determine the prevalence of genetic variants within any given study population and its association with disease. This is particularly important for the highly polymorphic genes that encode for enzymes which are involved in homocysteine metabolism. For example at least 60 polymorphisms have been described for the cystathione B-synthase gene.[13,15]

The present study found no association between the MTHFR C–>T677, CBS T–>C833, or the CBS G–>A919 polymorphisms and myocardial infarction in African-Americans. Our findings regarding MTHFR C–>T677 and myocardial infarction are in agreement with some but not all studies performed in Caucasians populations.[25,26,27] Similarly, our findings of no significant association regarding the CBS T–>C833 polymorphism and coronary artery disease/myocardial infarction are in agreement with those of other studies.[15,16,17] Though neither one of these polymorphisms was independently associated with increased risk of myocardial infarction, we performed an analysis designed to detect an interactive effect of carriership of dual mutations. The results of this analysis did not reveal an increased risk of myocardial infarction for those with both MTHFR C–>T677 and CBS T–>C833 mutations (heterozygote or homozygote) compared to those with neither of these mutations (P = .76). Since coronary artery disease/MI represents a polygenic disease, it is imperative for investigators to ascertain whether there are gene-gene interactions. Although we found no evidence of gene-gene interactions with these two polymorphisms in our study population, Botto et al[28] in the analysis of these same two genes found that while MTHFR C677T but not CBS T833C homozygosity was associated with a 2-fold risk for neural tube

defects, the interaction between the these two genes in the same individual resulted in a 5-fold increase risk in neural tube defects. This observation of Botto and colleagues not only demonstrates the importance of investigating gene-gene interactions, but also underscores the molecular complexity of homocysteine metabolism.

The prevalence of the MTHFR C–>T677 in our African-American controls was 17.3%, (allelic frequency of 10%), which consistent with that found in other African-American populations and is lower than that found in Caucasians.[8,9,10,29,30] The prevalence of the CBS T–>C833 polymorphism in our African-American controls was 37% (32% heterozygous and 5% homozygous) resulting in an allelic frequency of 21%. This same allelic frequency is also true for 844ins68 since it is always found with the T->C mutation. This finding in African-Americans was consistent with the findings of 37.7% heterozygosity and 4% homozygosity as reported by Franco et al[29] for that population. DNA analysis from the174 newborns revealed the allelic frequency for the CBS T–>C833 polymorphism to be 21.3% in African-Americans and 6.9% in white infants. Thus, the prevalence of the C allele in African-American infants is about 3-fold the prevalence of the allele among Caucasian infants, a statistically significant difference ($p<.001$). The birth prevalence is similar to that in the adult control group further suggesting that this polymorphism is not related to poor survival into middle and older age in African-Americans. Heterogeneity of the polymorphism has also been observed within the same population but who resided in different geographic regions.[20] This mutation has not been found among Asians and Amerindians.[31,32]

The CBS 919 polymorphism was not present among any of the study groups. 100% of the MI cases and control subjects as well as 100% of our African-American infants had the homozygous G/G genotype of the CBS 919 polymorphism. The prevalence of this allele was also nonexistent among our

white infants.

Although we were unable to measure plasma homocysteine levels, it has been reported that the MTHFR C677T, 833T->C/844ins68 polymorphisms together with A2756G polymorphism of the methionine synthase gene can contribute to the regulation of plasma homocysteine levels.[17,18] Although it is important to determine the polymorphic profile of genes involved in homocysteine metabolism for any given population, it is equally important to measure homocysteine plasma levels. Since gene frequencies vary among populations, it can be argued that the influence of genetic polymorphisms on the regulation of plasma homocysteine levels will also vary. Likewise the gene-environment interaction may differ among populations. Delineation of these parameters will be crucial to the understanding of homocysteine metabolism as it relates to cardiovascular disease.

In summary, our case-control study provides no evidence linking the MTHFR C–>T677, CBS G–>A919, or CBS T–>C833 polymorphisms to myocardial infarction in African-Americans. However, the racial differences of the CBS T–>C833 polymorphism suggest that further investigation into the other areas of the CBS gene is needed to further our understanding of possible links to vascular disease in African-Americans.

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Table 1. Distribution of cases and controls according to selected characteristics

Characteristic	Controls n=185		Cases n=110	
Mean age	55		56	
	n	**%**	**n**	**%**
Sex				
Male	91	49	72	66
Female	94	51	38	44
History of hypertension*				
No	63	34	15	14
Yes	122	66	94	86
Family history of vascular disease†			79	
No	106	57	37	34
Yes	79	43	73	66
Smoking				
Current	58	31	29	26
Former	45	24	62	56
Never	82	44	19	17

*One case did not know history of hypertension

†A history of blood clots, stroke, or heart attack

Table 2. **Frequency of the MTHFR C–>T677 and CBS T–>C833 polymorphisms and the odds ratios for myocardial infarction in African-American myocardial infarction patients and controls**

Genotype	Controls		MI Cases		Odds Ratio	95% CI	P
	n	%	n	%			
MTHFR C–>T677							
C/C	153	83	91	83		REFERENT	
C/T	28	15	17	15	1.0	0.52 - 2.0	.95
T/T	4	2	2	2	0.8	0.15 - 4.7	.84
CBS T–>C833*							
T/T	108	63	64	60		REFERENT	
T/C	56	32	36	34	1.1	0.64 - 1.8	.76
C/C	8	5	7	6	1.5	0.51 - 4.3	.47

MTHFR C–>T677 Recessive Model C/C vs. C/T and T/T:
odds ratio = 1.0; 95% CI 0.53 - 1.9; P = .99

CBS T–>C833 Recessive Model T/T vs. T/C and C/C:
odds ratio = 1.1; 95% CI 0.69 - 1.9; P = .62

*Genetic analysis for CBS T–>C833 unavailable for 13 controls and 3 cases